Larval Assay Section

1. Why larval mortality is an important measurement

Although we do not fully understand how a bee mortality of 50% in a cage study translates to its impact at a colony level, we nevertheless accept that adult mortality studies are important and required for assessment. On the other hand, we automatically assume that a 50% larval mortality does not have the same impact as a 50% adult mortality, to a colony. In this section, we argue that larval mortality is almost as important as adult mortality; in special cases it can be more important.

This is because not all adult workers are made equal and their contribution to the colony decrease with age. Fig. x shows that the value of an adult worker bee to the colony decreases with time. Assuming an average of summer adult longevity of 40 days, a worker at age 20 really only has half of its "value" left to the colony (because it already used half its useful life). In other words, a chemical that kills 10% of workers (that are all at 20 days old) really removes only 5% of the total effective foragers, from the colony's point of view in terms of time left to service the colony. A chemical that indiscriminately kills 20% of the foragers (of all ages), therefore, also only removes 10% of the "forage equivalence" (because the average of all foragers 1 to 40 days will be 20 days). This is assuming that there is an even distribution across all the age class of foragers, i.e. there is an equilibrium between the birth of newly emerged bees and the attrition rate of foragers, which is about 10% per day of all foragers (reference). A chemical that only kills 10% newly emerged bees, on the other hand, will remove 10% of foraging force, because these bees did not use up any of the 40 days of service yet for the colony. Here we ignore the fact that a 5 day old bee (a nurse) would be energetically more expensive than a forager (21-40 day old) because she has higher protein content in her head (larger hypopharyngeal glands) and higher fat content in her abdomen.

For the brood, a chemical that selectively kills sealed brood (a total of 11 days for workers), removes the same amount of forager-equivalence as is the case of 1 day old bees (i.e. 10% mortality removes 10% of forager-days), because these larvae/pupae already consumed the full energy (cost to create the biomass), yet they have not realized any days of the 40 days of service yet.

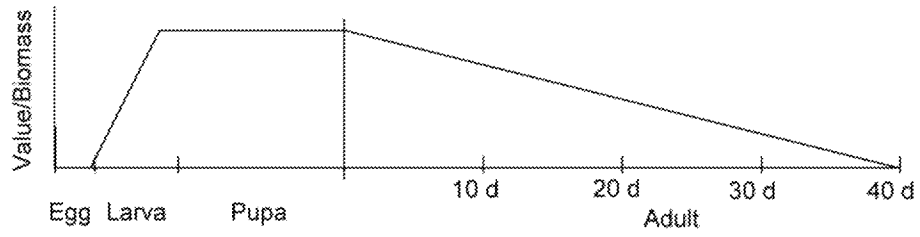

Fig. x. Value of brood (in terms of biomass) and workers (in terms of days of service left) to the honey bee colony. A straight line is used for increase of larval biomass, but in reality the weight increase should be exponential.

For eggs and larvae it is more complicated. From a pure energy cost of point of view, a 10% of mortality for 1 day old larvae is much less costly than 10% mortality for 5 day old larvae. Assuming that workers cannot "re-cycle" the proteins in the killed larvae, because they died from toxicity and workers might be able to detect this, then a newly hatched larva would be about 1,000 times less costly energetically than a mature larvae. This is because a newly hatched larva would weight similar to an egg, which would be around 0.12 mg, while a mature larva weighs around 155 mg (~1,000 x difference, ref). We do not know if the queen can compensate by laying more eggs if 20% of eggs or young larvae is killed. But even if she could, there is still a real cost to the colony by killing the larvae due to the energy invested to grow the larvae. Four day old larvae weigh about 100 mg each, thus a 50% mortality at this stage would be equivalent to removing 32% (0.5 x 100/155) of mature larvae, which is also equivalent to removing 32% of 1 day old foragers.

Besides the energetic cost to the colony, there will also be impact on the colony strength. In other words, even if the cost to rear larvae is zero, removing 50% of larvae will still impact the colony because not enough workers will be present to build a strong colony, make honey, and survive the winter. How the impact of removing varying amounts of larvae translates to colony increase will require modeling. We recommend modifying an existing model (such as Beepop) to determine how larval mortality will affect colony strength at different times of the year.

In conclusion, brood mortality can be important to the colony, even though it is not as straight forward as worker mortality. Even for adult mortality is not as straight forward as on the surface, after we incorporated the concept of "days of service" as presented in Fig. x. Our exercise here also suggests that adult mortality should be subdivided into foragers or nurses, as these two groups have different values to the colony, in addition to their vastly different physiology which should cause differential resistance to chemicals.

2. Description of the method

   Aupinel method of larval rearing – this will include clarity on issues that must remain as reported in the method (such as the rearing temperature) or those for which some flexibility is allowed (for example: one does not need to use a paint brush to transfer larvae – any grafting device will do).

   Possible improvements.

3. Defined and defended endpoints

   - Larvae (from grafting until defecation): NOEC/LC_{50} and weight at defecation
   - Pupae (from defecation to adult emergence): NOEC/ LC_{50} and weight at emergence
   - Adult (from emergence until mortality): NOEC/LC_{50}

   Commented [WU1]: Should we do mortality or just include an endpoint? Say, day 18 or 22?

4. Determining the quality of the test method

   - % untreated larvae to defecation ≥90%
   - % untreated larvae to adulthood ≥ 80%
   - ≤ 20% mortality of resulting adult bees on day 18 (Huang) or 22 (others?)

5. Gaps in knowledge/future research

   - Screening royal jelly for pesticides, antibiotics, and pathogens (what are acceptable levels of each).
   - ***The need for a chemically defined diet.***

   Because royal jelly (RJ) is costly to produce locally, imported RJ from Asia is often used. These RJ usually have higher levels of pesticides, and may contain exotic honey bee viruses, bacteria, and other pathogens. Gamma irradiation can be used to remove the pathogens, however it is not clear whether pesticide levels would be decreased after treatment. In addition, it will be difficult to have one single RJ supplier to provide RJ to test labs around the world. OEDC specifically requires more than one supplier of chemicals or test materials. Even if that single supplier exists and is allowed by OEDC, it lacks year to year RJ consistency because pesticide levels and pathogen levels might change year to year in the production colonies. It is therefore vitally important to have a chemically defined diet to mitigate these drawbacks.
   A chemically defined diet should be possible, as larvae can not only survive, but also experience weight gain in a similar fashion to RJ based diet (Huang, unpublished results). These larvae survive for 3-4 days but failed to defecate or pupate in the test system. It appears that RJ major proteins might not as important as we thought. By adding more ingredients to the present media, one should be able to eventually produce adults.

- Discuss the correct protocol for method modification (ie., if others find ways to increase throughput, increase bee survival (any stage), and/or decrease test cost).
  Significant improvements can be posted periodically at [HYPERLINK "http://larvae.bees.net"], but how can the modified method be incorporated into the "method" accepted by the regulating agencies?
- How to properly ring test the method
- What are acceptable differences between labs:
- Can/should this method be used to investigate sublethal effects of pesticides?